A New Pregnane and a New Diphenylmethane from the Root Barks of *Periploca sepium*

by **Lei Wang**[a]), **Zhi-Qi Yin**[a]), **Wen-Bin Shen**[b]), **Qing-Wen Zhang**[c]), and **Wen-Cai Ye***[a])[d])

[a]) Department of Phytochemistry, China Pharmaceutical University, Nanjing 210038, P. R. China (phone: +86-25-8532-2132; fax: +86-25-8530-1528; e-mail: chywc@yahoo.com.cn)

[b]) Center of Instrumental Analysis, China Pharmaceutical University, Nanjing 210009, P. R. China

[c]) Institute of Chinese Medical Sciences, University of Macau, Taipa, Macau, P. R. China

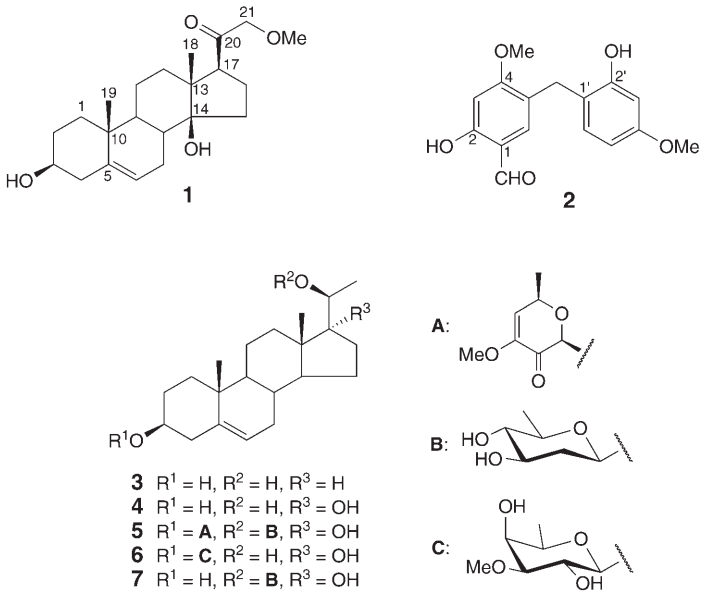
[d]) Institute of Traditional Chinese Medicine and Natural Products, Jinan University, Guangzhou 510632, P. R. China

A new polyoxygenated pregnane genin and a new diphenylmethane, along with five known pregnane derivatives, were isolated from the root barks of *Periploca sepium*. The structures of the new compounds were elucidated as (3β,14β)-3,14-dihydroxy-21-methoxypregn-5-en-20-one (**1**) and 2-hydroxy-5-(2-hydroxy-4-methoxybenzyl)-4-methoxybenzaldehyde (**2**) on the basis of spectroscopic methods, especially 2D-NMR and MS analyses. The known compounds were identified by comparing their physical and spectroscopic data with those reported in the literature.

Introduction. – The root bark of *Periploca sepium* BGE. (Asclepiadaceae), a well known traditional Chinese medicine called 'xiangjiapi', has been widely used in the treatment of autoimmune diseases, especially for rheumatoid arthritis [1]. Previous phytochemical studies of this plant, mainly carried out by some Japanese research groups, have led to the isolation of more than 32 pregnane derivatives, 4 cardenolides, and 4 oligosaccharides [2]. In addition, some interesting pharmacological activities have been reported. Periplocoside A had significant antitumor activity [3], and some pregnane glycosides showed differentiation inducing activities on mouse myeloid leukemia cells [4]. Recently, periplocoside E was reported as an immunosuppressant which could directly suppress T-cell activation *in vitro* and *in vivo* [5].

In searching for bioactive constituents from the root barks of *Periploca sepium*, we herein describe the isolation and structure elucidation of a new polyoxygenated pregnane genin, (3β,14β)-3,14-dihydroxy-21-methoxypregn-5-en-20-one (**1**), and of a new diphenylmethane, 2-hydroxy-5-(2-hydroxy-4-methoxybenzyl)-4-methoxybenzaldehyde (**2**). Their structures were elucidated on the basis of spectroscopic methods, especially 2D-NMR techniques, including ^{1}H,^{1}H-COSY, ROESY, HMQC, and HMBC experiments. In addition, five known pregnane derivatives (3β,20*S*)-pregn-5-ene-3,20-diol (**3**), (3β,17α,20*S*)-pregn-5-ene-3,17,20-triol (**4**), periploside B (**5**), periplocoside L (**6**), and periplocoside N (**7**) were also isolated from this plant and identified by comparing their physical and spectroscopic data with those reported in the literature.

Results and Discussion. – The pulverized, air-dried root barks of *P. sepium* were extracted with 70% EtOH. The residue of the extract was suspended in H_2O and then



successively extracted with AcOEt and BuOH. Extensive purification of the AcOEt extract by repeated column chromatography finally afforded compounds **1**–**7**.

Compound **1** was obtained as a colorless, amorphous, optically active powder. The HR-ESI-MS of **1** exhibited a quasimolecular ion $[M+\mathrm{Na}]^+$ at m/z 385.2339, consistent with the molecular formula $C_{22}H_{34}O_4$. The IR spectrum revealed the presence of OH (3456 cm^{-1}) and C=O (1729 cm^{-1}) groups. From the ^{1}H- and ^{13}C-NMR (*Table 1*), DEPT, HMBC (*Fig. 1*), and ROESY (*Fig. 1*) data, the structure of **1** was established as (3β,14β)-3,14-dihydroxy-21-methoxypregn-5-en-20-one.

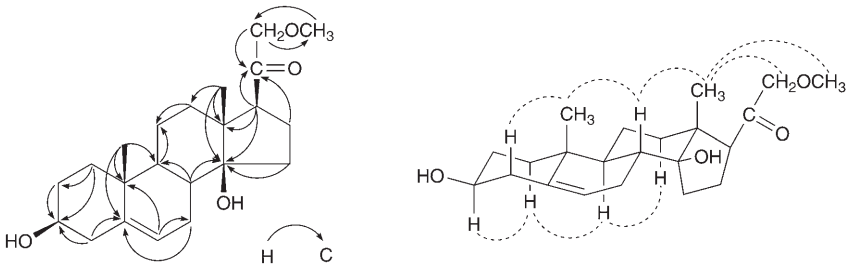


Fig. 1. *Key HMBC* (left) *and selected key ROESY* (right) *correlations of* **1**

The ^{1}H-NMR spectrum of **1** displayed signals for two Me groups at δ(H) 0.98 and 0.99 (2*s*), a MeO group at δ(H) 3.43 (*s*), and an olefinic H-atom at δ(H) 5.40–5.41 (*m*). A total of 22 C-signals were observed in the ^{13}C-NMR and DEPT spectra of **1**, with one C=O group, four quaternary C-atoms, and three Me, nine CH_2, and five CH groups. These data indicated the presence of a C_{21}-steroid skeleton. Comparison of the ^{13}C-NMR data of **1** with those of the known compound (3β,5β,14β)-3,14-dihydroxy-

Table 1. *^{1}H- and ^{13}C-NMR Data of Compound* **1**. δ in ppm, J in Hz.

	δ(C)[a])	δ(H)[b])		δ(C)[a])	δ(H)[b])
H_α–C(1)	37.29	1.09 (*td*, J = 12.9, 3.5)	H_α–C(12)	38.50	1.49–1.55 (*m*)
H_β–C(1)		1.82–1.89 (*m*)	H_β–C(12)		1.36–1.42 (*m*)
H_α–C(2)	31.56	1.82–1.88 (*m*)	C(13)	49.39	–
H_β–C(2)		1.47–1.55 (*m*)	C(14)	85.12	–
H_α–C(3)	71.65	3.48–3.55 (*m*)	H_α–C(15)	34.48	1.83–1.89 (*m*)
H_α–C(4)	42.13	2.29–2.33 (*m*)	H_β–C(15)		2.10–2.15 (*m*)
H_β–C(4)		2.23 (br. *t*, J = 12.2)	H_α–C(16)	24.47	1.85–1.91 (*m*)
C(5)	139.04	–	H_β–C(16)		1.95–2.02 (*m*)
H–C(6)	122.24	5.40–5.41(*m*)	H_α–C(17)	57.17	2.93 (*dd*, J = 9.9, 4.2)
H_α–C(7)	27.35	1.83–1.89 (*m*)	Me(18)	14.96	0.98 (*s*)
H_β–C(7)		2.33–2.38 (*m*)	Me(19)	19.42	0.99 (*s*)
H_β–C(8)	36.32	1.76 (*td*, J = 11.8, 5.2)	C(20)	216.18	–
H_α–C(9)	45.92	1.17 (*td*, J = 11.8, 4.3)	CH_2(21)	78.93	4.05 (*d*, J = 18.1),
C(10)	36.84	–			4.10 (*d*, J = 18.1)
H_α–C(11)	20.80	1.46–1.52 (*m*)	MeO	59.29	3.43 (*s*)
H_β–C(11)		1.40–1.45 (*m*)			

[a]) Measured at 125 MHz in $CDCl_3$. [b]) Measured at 500 MHz in $CDCl_3$.

21-methoxypregnan-20-one [6] revealed that the signals were similar, except for the appearance of the signals for a C=C bond (δ(C) 139.04 and 122.24) of **1** and the disappearance of the signals for C(5) (δ(C) 36.5) and C(6) (δ(C) 26.7) of the known compound. Thus, **1** was proposed to be a 5,6-didehydro derivative of (3β,5β,14β)-3,14-dihydroxy-21-methoxypregnan-20-one, which was supported by the ^{13}C,^{1}H-HMBC C(5)/Me(19), CH_2(4), and CH_2(7) (*Fig. 1*). The configuration assignments of the CH and nonequivalent CH_2 protons were achieved with the aid of the ROESY data (*Fig. 1*), in a similar way as with (11α,12β)-11-*O*-tigloyl-12-*O*-acetyltenacigenin B [7] (tenacigenin B = (3β,5α,11α,12β,14β,17α)-8,14-epoxy-3,11,12-trihydroxypregnan-20-one). H–C(3) was assigned to be in the relative α-configuration (axial orientation) according to the J(H–C(3),H_β–C(4)) value (12.2 Hz). The splitting pattern and coupling constants of the signal of H–C(17) (δ(H) 2.93 (*dd*, J = 9.9, 4.2 Hz)) suggested that the side chain at C(17) of **1** was β-oriented [7], as confirmed by the correlation between Me(18) and CH_2(21) in the ROESY plot.

It is noteworthy that the possible role of a 21-methoxypregnane is a storage form of a 21-hydroxypregnan-20-one derivative [6]. Thus, compound **1** is obviously biogenetically related to (3β,5β,14β)-3,14,21-trihydroxypregnan-20-one, which had been established to be a precursor of cardenolides [8]. The presence of compound **1** could suggest the pregnane pathway for the biosynthesis of cardenolides in this plant.

Compound **2** was isolated as colorless needles. The molecular formula of compound **2** was determined to be $C_{16}H_{16}O_5$ by HR-ESI-MS (*m*/*z* 289.1090 ([*M* + H]$^+$)). The full assignments of ^{1}H- and ^{13}C-NMR signals (*Table 2*) of **2** were accomplished by a combination of HMQC, HMBC (*Fig. 2*), and ROESY (*Fig. 2*) data, which allowed to elucidate the structure of **2** as 2-hydroxy-5-(2-hydroxy-4-methoxybenzyl)-4-methoxybenzaldehyde.

The ^{1}H-NMR spectrum of **2** indicated signals for two MeO groups (δ(H) 3.76 and 3.96 (2*s*)), two OH groups (δ(H) 5.77 and 11.40 (2*s*)), a CHO group (δ(H) 9.66 (*s*)), and five aromatic protons (δ(H) 6.43 (*d*,

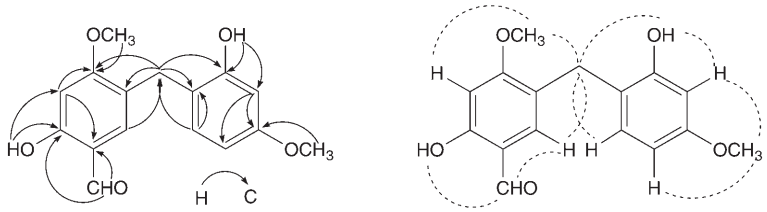


Fig. 2. *Key HMBC* (left) *and key ROESY* (right) *correlations of* **2**

Table 2. *^{1}H- and ^{13}C-NMR Data of Compound* **2**. δ in ppm, J in Hz..

	δ(C)[a])	δ(H)[b])		δ(C)[a])	δ(H)[b])
CHO	194.57	9.66 (*s*)	C(1′)	118.09	–
C(1)	115.04	–	C(2′)	154.67	–
C(2)	163.37	–	H–C(3′)	102.10	6.43 (*d*, *J* = 2.5)
H–C(3)	99.15	6.45 (*s*)	C(4′)	159.84	–
C(4)	163.37	–	H–C(5′)	106.63	6.46 (*dd*, *J* = 8.3, 2.5)
C(5)	121.64	–	H–C(6′)	131.15	7.06 (*d*, *J* = 8.3)
H–C(6)	134.47	7.24 (*s*)	*Me*O–C(4′)	55.33	3.76 (*s*)
CH$_2$–C(5)	28.79	3.79 (*s*)	OH–C(2)	–	11.40 (*s*)
*Me*O–C(4)	56.25	3.96 (*s*)	OH–C(2′)	–	5.77 (*s*)

[a]) Measured at 125 MHz in $CDCl_3$. [b]) Measured at 500 MHz in $CDCl_3$.

J = 2.5 Hz), 6.45 (*s*), 6.46 (*dd, J* = 8.3, 2.5 Hz), 7.06 (*d*, *J* = 8.3 Hz), and 7.24 (*s*)). The ^{13}C-NMR and DEPT spectra of **2** exhibited 16 C-signals attributed to two Me, one CH_2, one CH(=O), and five aromatic CH groups, and to seven quaternary aromatic C-atoms, which suggested that **2** possessed two aromatic rings substituted by the above-mentioned functional groups. Furthermore, the signals due to the CH_2 group (δ(H) 3.79 (*s*); δ(C) 28.79) indicated that the two aromatic rings were connected by a CH_2 group [9], which was confirmed by the ^{1}H,^{13}C long-range correlations CH_2–C(5)/C(4), C(5), C(1′), and C(2′). In the ^{1}H,^{13}C-HMBC plot of **2**, the following correlations were observed: MeO–C(4)/C(4), OH–C(2)/C(2) and C(3), CHO/C(1) and C(2), OH–C(2′)/C(2′) and C(3′), MeO–C(4′)/C(4′), H–C(6)/*C*H$_2$–C(5), and H–C(6′)/*C*H$_2$–C(5) (*Fig. 2*). Furthermore, the location of the substituents at the aromatic rings were deduced by the ROESY experiment (*Fig. 2*). The correlations MeO–C(4)/CH_2–C(5) and H–C(3) confirmed the position of this MeO group at C(4). Similarly, the NOEs OH (δ(H) 5.77)/CH_2–(5) and H–C(3′), as well as MeO–C(4′)/H–C(3′) and H–C(5′) confirmed that the OH was attached to C(2′) and the MeO to C(4′). In addition, the CHO and OH (δ(H) 11.40) were located at C(1) and C(2), respectively, according to the correlations CHO/H–C(6) and OH (δ(H) 11.40)/CHO.

The five known compounds (3β,20*S*)-pregn-5-ene-3,20-diol (**3**) [10], (3β,17α,20*S*)-pregn-5-ene-3,17,20-triol (**4**) [11], periploside B (**5**) [12], periplocoside L (**6**) [11], and periplocoside N (**7**) [12] were also isolated and identified on the basis of their physical and spectroscopic data.

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Experimental Part

General. Column chromatography (CC): Silica gel (200–300 mesh), *H60* (*Qingdao Marine Chemical Plant*, Qingdao, P. R. China); *Sephadex LH-20* (*Pharmacia*). TLC: precoated silica gel GF_{254} plates (*Qingdao Marine Chemical Plant*, Qingdao, P. R. China). M.p.: *XT-4* micro-melting-point apparatus; uncorrected. Optical rotation: *Jasco-P-1020* polarimeter. UV Spectra: *Shimadzu-UV-2450* UV/VIS spectrophotometer; λ_{max} (log ε) in nm. IR Spectra (KBr): *Bruker-Tensor-27* FT-IR spectrometer; in cm^{-1}. ^{1}H-, ^{13}C-, and 2D-NMR Spectra: *Bruker-AV-500* spectrometer; δ in ppm rel. to Me_4Si, *J* in Hz. MS: *Agilent-1100-LC/MSD-Trap* (ESI-MS) and *Micro-Q-TOF* (HR-ESI-MS) spectrometer; in *m*/*z*.

Plant Material. The root barks of *Periploca sepium* were purchased from Xinjiang Uygur Autonomous Region, P. R. China, in April 2004, and identified by Prof. *Min-Jian Qin* (China Pharmaceutical University). A voucher specimen (No. 20040518) was deposited in the herbarium of the China Pharmaceutical University, Nanjing, P. R. China.

Extraction and Isolation. The air-dried root barks of *P. sepium* (10 kg) were pulverized and extracted with 70% EtOH (3×). The extract was concentrated to a suitable volume, suspended in H_2O, and then successively extracted with AcOEt and BuOH. The AcOEt extract was concentrated to afford a residue (190 g), which was further separated by CC (SiO_2, $CHCl_3 \rightarrow CHCl_3$/MeOH 1:1): *Fractions A–F. Fr. B* (70 g) was subjected to CC (SiO_2, petroleum ether/Me_2CO 50:1 → 1:1): *Fr. B.1–B.5. Fr. B.1* (5.5 g) was purified by CC (SiO_2, petroleum ether/Me_2CO 100:1 → 5:1), and the resulting major component was purified by CC (*Sephadex LH-20*, $CHCl_3$/MeOH 1:1): **2** (12 mg). *Fr. B.2* (9 g) was separated by CC (SiO_2, petroleum ether/Me_2CO 20:1 → 5:1): *Fr. B.2.1* and *Fr. B.2.2. Fr. B.2.1* (105 mg) was resubjected to CC (*Sephadex LH-20*, $CHCl_3$/MeOH 1:1): **1** (23 mg). *Fr. B.2.2* (190 mg) was also purified by CC (*Sephadex LH-20*, $CHCl_3$/MeOH 1:1): **3** (45 mg). *Fr. B.3* (12 g) was subjected to CC (SiO_2, petroleum ether/Me_2CO 10:1 → 2:1): **4** (33 mg) and **6** (58 mg). *Fr. C* (11 g) was separated by CC (SiO_2, $CHCl_3$/MeOH 100:1 → 10:1): *Fr. C.1* and *Fr. C.2. Fr. C.1* (130 mg) was purified by CC (*Sephadex LH-20*, $CHCl_3$/MeOH 1:1): **5** (16 mg). *Fr. C.2* (2.2 g) was subjected to CC (SiO_2, $CHCl_3$/MeOH 20:1): **7** (20 mg).

(3β,14β)-3,14-Dihydroxy-21-methoxypregn-5-en-20-one (**1**): Colorless, amorphous powder. M.p. 177–179° (AcOEt). $[\alpha]_D^{25} = 10.9$ ($c = 0.09$, $CHCl_3$). IR (KBr): 3456, 2927, 2854, 1729, 1383, 1278. ^{1}H- and ^{13}C-NMR: *Table 1*. ESI-MS (pos.): 385.2 ($[M+Na]^+$), 747.1 ($[2M+Na]^+$). HR-ESI-MS: 385.2339 ($[M+Na]^+$, $C_{22}H_{34}O_4Na^+$; calc. 385.2355).

2-Hydroxy-5-(2-hydroxy-4-methoxybenzyl)-4-methoxybenzaldehyde (**2**): Colorless needles (MeOH). M.p. 168–169° (MeOH). UV ($CHCl_3$): 242 (4.11), 279 (4.08), 328 (3.62). IR (KBr): 3432, 1644, 1623, 1592, 1280, 1251, 1165, 1129, 783. ^{1}H- and ^{13}C-NMR: *Table 2*. ESI-MS (neg.): 287 ($[M-H]^-$). HR-ESI-MS: 289.1090 ($[M+H]^+$, $C_{16}H_{17}O_5^+$; calc. 289.1076).

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